Available online on 20.06.2019 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

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Simultaneous Estimation & Validation of Praziquantel & Pyrantel Pamoate in Bulk & Pharmaceutical Dosage Form by Using RP-HPLC

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ABSTRACT

The first reversed phase high performance liquid chromatographic method for quantitation studies of, Praziquantel and Pyrantel Pamoate has been developed and validated to be a simple, sensitive, rapid, specific, precise, and accurate method. Chromatographic separation was achieved on C18 column (250×4.6 mm-5µm p.s). Methanol and water in a ratio [85:15 v/v] as a mobile phase at flow rate of 0.8ml/min. UV detection was operated at 217 nm and injection volum was 20 µl.. The proposed method showed good linearity, accuracy, precision and was successfully applied for determination of the drugs in laboratory prepared pharmaceutical dosage forms. The current method has been statistically validated according to the ICH guidelines and this method has been subsequently developed and applied successfully to determine the levels of Praziquantel and Pyrantel pamoate in a combined formulation and in the routine quality control analysis with good accuracy and sensitivity.

Keywords: Praziquantel, Pyrantel pamoate, Quantitation Studies, RP-HPLC ,

Article Info: Received 03 May 2019; Review Completed 05 June 2019; Accepted 10 June 2019; Available online 20 June 2019

Cite this article as:

Dighe MS, Shinde GS, Dhamak KV, Shete RG, Simultaneous Estimation & Validation of Praziquantel & Pyrantel Pamoate in Bulk & Pharmaceutical Dosage Form by Using RP-HPLC, Journal of Drug Delivery and Therapeutics. 2019; 9(3-s):688-692

http://dx.doi.org/10.22270/jddt.v9i3-s.2951

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INTRODUCTION

The high performance liquid chromatography of praziquantel and pyrantel pamoate in tablet dosage forms of total .[1] Helmint infections which are influceneed by parasites ,affect more than one billion people in the world .owing to the narrow spectrum of antihelmintic drugs it is needed to use combination chemotheraphy to control mixed infections. are the most common treatments of helmint infections .Another drug combination ,which is the theme of our work ,consists of oxantel pamoate ,pyrantel pamoate and praziquantel and notably is being used to treat dogs · determined praziquantel by using C^{18} column at 217 nm after solid phase extraction for preparing the sample . enatiomers of praziquantel in human plasma were separated by Liu and Stewart by using cellulose –based chrial column and Vu detrctor. Binary mixture and combined preparation of similar antihelmintic drugs as mebendazole,fenbendazole ,albendazole and their related impurities determined together .[2] Helminthiases are parasitic diseases commonly found in pets and cause significant morbidity in dogs and cats.These infections are promoted mainly for nematodes ,cestodes and trematodes and have public health significance because parasitic diseases also transmissible to humans [3]

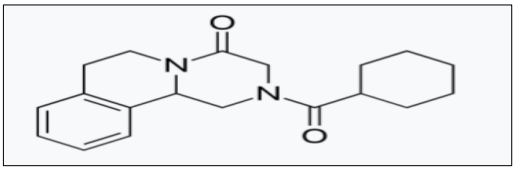

Fig1: Structure of Praziquantel

Pyrantel pamoate is an orally administered veterinary anthelmintic that is effective against a variety of round worms and hook worms in dogs ,cats ,horses ,birds and rabbits .It also used to treat pin worms in humans mechanism of action is drug exerts its action as a depolarizing blocking agent that particularly affords spastic paralysis in susceptible helminatic [4] This has the result of causing thr worm to lose its grip on the intestinal wall and be passed out of the system by natural process .since pyrantel is poorly absorbed by the hosts is unaffected by the small dosage of medication used . Spastic (tetanic) paralyzing agents. In particular pyrantel pamoate , may induce complete intestinal obstruction in heavy worm load. Giardia is a cosmopolitan parasite colonises the small intestine of domestic animals,inciuding liverstock ,dogs and cats .Giardia

is also a parasite of humans and wildlife ,due to the zoonotic risk to humans,it is very important to identify and treat giardiasis in dogs and cats [5]

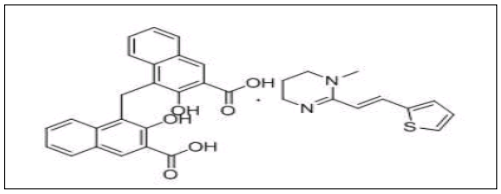

Fig2: Structure of Pyrantel pamoate

MATERIAL AND METHOD

List of Instruments

Table 1: List of apparatus/ instruments used.

Sr no	Name	Modele
1	Weighing balance	PGB 100
2	Ultra-Sonicator	WUC-4L
3	UV- Spectrophotometer and Software	UV2450 UV probe v 2.3.3
4	HPLC	HPLC 3000 series

List of Chemicals

Table 2: List of chemical used Preparation of mobile phase

Sr. No	Reagents and Chemicals	Details
1	Methanol	HPLC grade
2	Water	HPLC grade
3	Phosphoric acid	HPLC grade
4	Praziquantel	Veko Care pvt.Ltd
	Pytantel Pamoate	Veko Care pvt.Ltd

Mixed a HPLC grade Methanol: ortho phosphoric acid Buffer 10mM (80:20)in volumetric flask. Filter through undervaccum filtration.

Preparation Stock Solution:

Procedure:

Accurately weighed quantity of Praziquantel and Pyrantelpamoate 10 mg individually dissolved in 10 ml volumetric flask using mobile phase and solution was sonicated for 20 minutes and volume is make up to the mark to get 1000 µg/ml and filtered through 0.45µm membrane filter.

Preparation of Sample solution:

Procedure:

20 tablets were weighed and powdered, tablets powder equivalent to 10 mg of Praziquantel and Pyrantelpamoate was transferred 100 ml volumetric flask, sufficient amount of mobile phase was added and dissolved by 20minutes ultrasonication. then made the volume up to the mark with the mobile phase and filtered with 0.45µ filter paper. Pipette out from above solution and diluted to 10 ml mobile phase and use for sample injection

Selection of analytical wavelength:

Accurately weighed quantity of Praziquantel and Pyrantelpamoate 10 mg dissolved in 100 ml volumetric flask and volume is make up to the mark to get 100 µg/ml.. Solution was scanned using UV-Visible Spectrophotometer in the spectrum mode between the wavelength ranges of 400 nm to 200 nm. The wavelength selected was 217 nm.

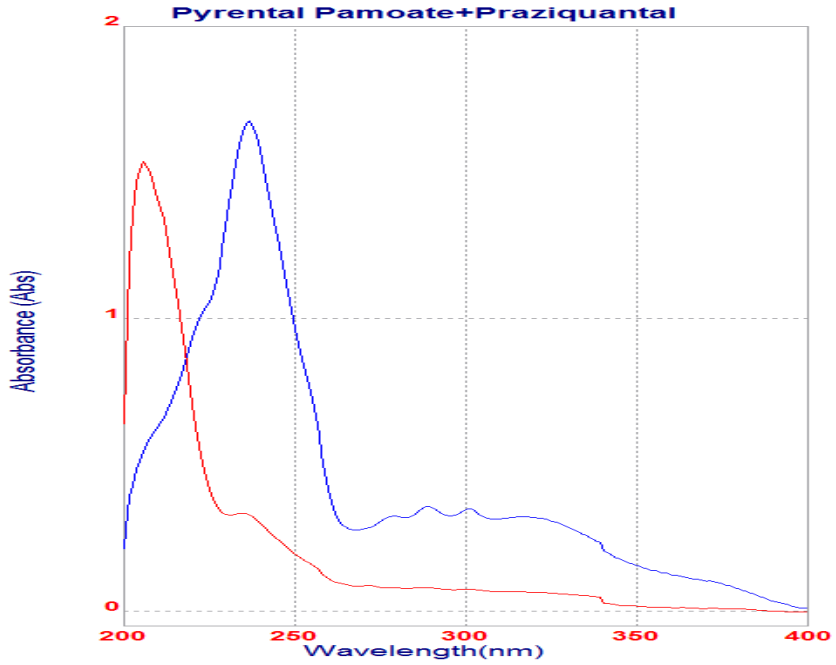

Fig. 3.Wavelength of Praziquantel and Pyrantelpamoate

Optimized chromatographic condition

In the present study the separation of Praziquantel and Pyrantel pamoate was achieved by using column cosmosil C18, (250×4.6mm,5µ)with mobile phase consisting of mixture of methanol and water in the ratio of 85:15 at a flow rate 0.8 ml/min with uv detection wavelength of 217nm at ambient temperature. The run time for praziquantel and pyrantel pamoate found to be 6.59min respectively.

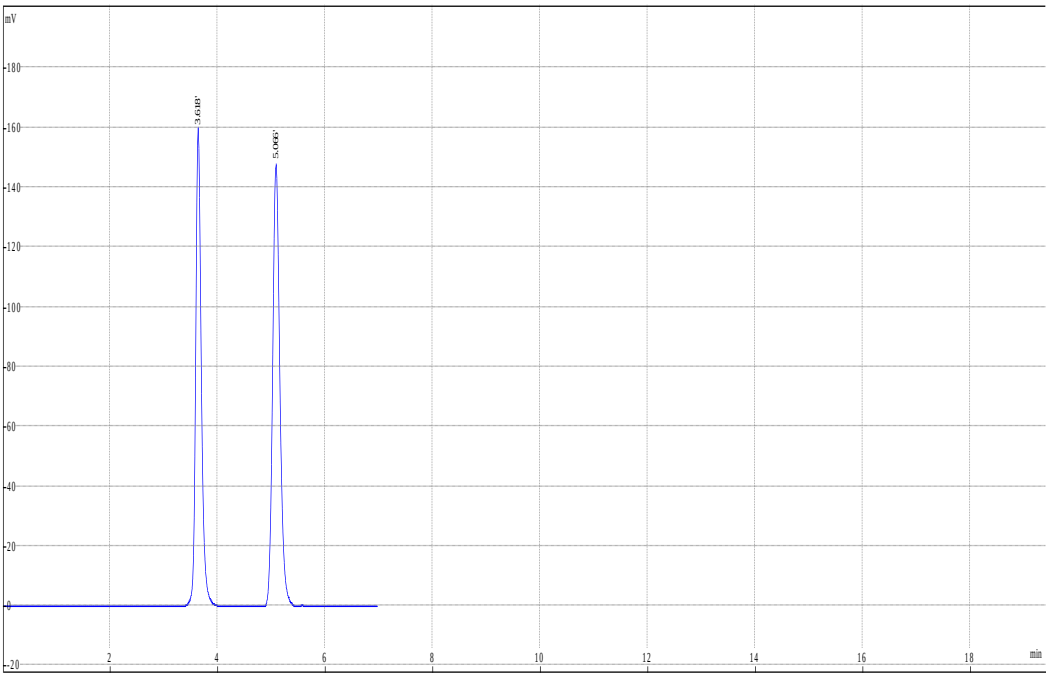

Figure No. 3. Chromatogram for sample solution.

Name of drug	Time	Area	Resolution	Th. Plate	Asymmetry
Praziquantel	3.618	1155711	6.56	7736	1.25
Pyrantelpamoate	5.066	1263705	0.00	6916	1.18

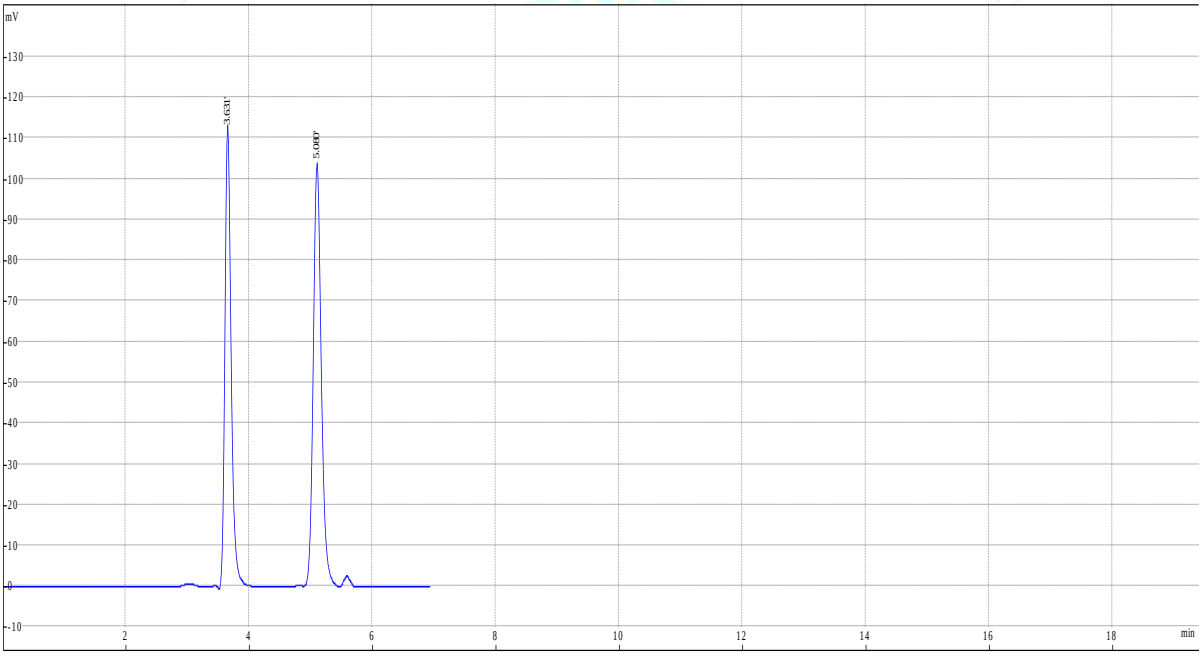

Figure No. 5. Chromatogram for standard solution.

Name	RT(min)	Area	Resolution	Theoretical plate	Asymmetry factor
Praziquantel	3.631	879928	6.57	7612	1.31
Pyrantelpamoate	5.080	980190	0.00	7143	1.17

Validation of the Developed Method:

A. Linearity:

Linearity of an analytical method is its ability to elicit test results that are directly proportional to the concentration of analyte in samples within a given range.The linearity of the analytical method is determined by mathematical treatment of test results obtained by analysis of samples with analyte concentrations across the claimed range. Area plotted graphically as a function of analyte concentration. Percentage curve fittings are calculated.

Table 4: Result of linearity

SI.NO.	Linearity Level	Praziquantel		Pyrentel Pamoate	
		Conc.(µg / ml)	Peak Area	Conc.(µg / ml)	Peak Area
1	Level-1	10	184335	10	215539
2	Level-2	20	406014	20	444221
3	Level-3	30	649335	30	714782
4	Level-4	40	886149	40	982534
5	Level-5	50	1149364	50	1256928
Linearity Range		1.30 -8.5		19.25 -116.4	
Slope		18861.4		21741.7	
R^2		0.999		0.998	

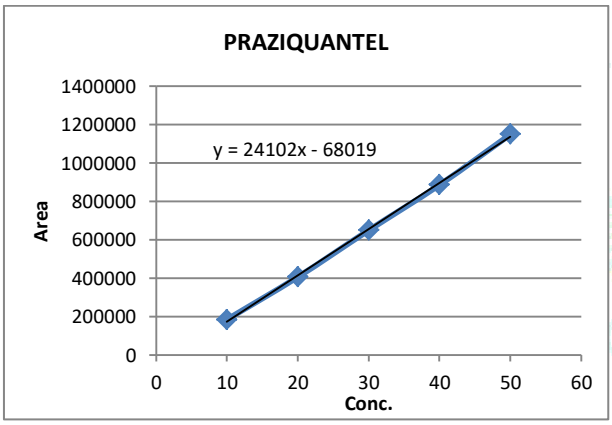

Fig.No. 6.Linearity graph for Praziquantel

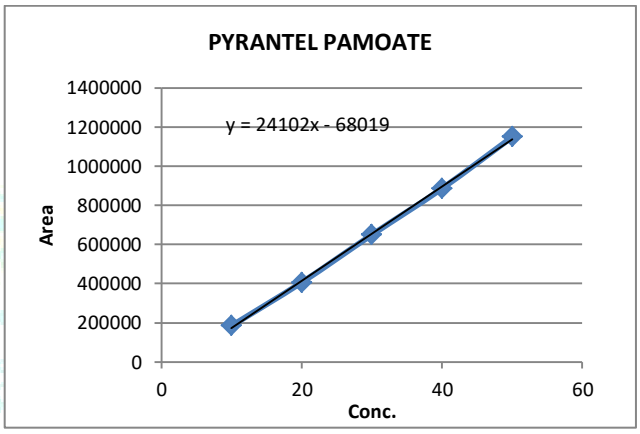

Fig.No. 7.Linearity graph for Pyrantel pamoate.

B. ACCURACY

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. Accuracy may often the expressed as percent recovery by the assay of known added amounts of analyte according to USP guidelines.The accuracy of an analytical method is determined by applying the method to analyzed samples, to which known amounts of analyte have been added. The accuracy is calculated from the test results as the percentage of analyte recovered by the assay.

Table 5: Recovery Studies for Praziquantel and Pyrantel Pamoate

Drug	Accuracy level	Area of Standard	Area of Sample	% Recovery
Praziquantel	50	705248	825248	**105.2**
	100	979423	1029423	**108.2**
	150	1305721	13957215	**106.3**
Pyrantel pamoate	50	645948	707095	109.4662419
	100	879928	980190	111.3943414
	150	1155711	1263705	109.3443776

C. Robustness:

The robustness of an analytical method is determined by analysis of aliquots from homogenous lots by differing physical parameters that may differ but are still within the specified parameters of the assay. The sample along with standard was injected under different chromatographic conditions as shown below

Table 6: Robustness Result Data

Sr.no	Conc	Peak area	Peak Area
		Praziquantel	Pyrantel Pamoate
1	25	406014	406014
2	25	405186	409524
3	25	405945	407079
Mean		405715	407539
Standard Deviation		459.42	1799.65
% RSD		0.1132	0.4415

Precision: Precision of the method was studied as repeatability and intermediate precision .Repeatability was determined by system or instrumental and method precision (intra assay precision). System precision studies were performed by injecting six repeated injection of 100 % concentration from standard solution within a day. Peak area and % RSD were calculated. Method precision studies were performed and three replicates were obtained. The % RSD values are found to be within the limits.

Intermediate Precision: The intermediate precision of the method was checked by intra –day and intra –day study and results

Table 7: Method Precision Results for Praziquantel and Pyrantel Pamoate

Replicates	Peak Area		Con c .Obtained (µg /ml)	
	Praziquantel	Pyrantel Pamoate	Praziquantel	Pyrantel Pamoate
1	646865	748447	9.745	9.845
2	648675	718447	9.643	9.96
3	650465	713625	10.4	10.12
Mean	647268	715386	9.89	9.99
Standard Deviation			0.2839	0.2936
% RSD			1.68	1.24

Table 9 Inter –Day Precission Results for Praziquantel and Pyrantel Pamoate

Replicate	Date Interval	Praziquantel		Pyrantel Pamoate	
		Peak Area	Conc.	Peak Area	Conc.
1	Day 1	651713	9.84	715732	9.97
2	Day 2	647268	9.93	719485	9.94
3	Day 3	642754	9.78	712931	9.98
Mean			9.83		9.96
Standard deviation			0.246		0.178
% RSD			1.60		0.30

CONCLUSION

The proposed Quantitation studies and validation method was found to be simple, precise, accurate and rapid for the determination of Praziquantel and Pyrantel pamoate. The coefficient of correlation was obtained in acceptable range .The percentage recovery obtained in acceptable range .variation in flow rate, wavelength, does not have any effect on the % RSD of standard and assay value.The relative standard deviation of main peak area ,tailing factor and therotical plate is well within the acceptable range . Hence the precision of given method is confirmed. Thus from the above result of the individual method is conclude that the analytical method is validated and found to be satisfactory.

Acknowledgment: The authors express their gratitude to the Pravara Rural College of Pharmacy,Loni and a division of Veko care Pharma Limited, Pune for providing the gift sample of Praziquantel and Pyrantel pamoate.

Conflict of Interest: The authors declare no conflict of interest.

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